Overcoming Double-Poisson Limitation for Co-encapsulation in Droplets through Hydrodynamic Close Packing of Cells

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ABSTRACT

The study of cell-cell interaction in high-throughput is critically important in many biological systems, including oncology, immunology, and tissue engineering. However, the passive co-encapsulation of one type A cell and one type B cell per single droplet, termed 1-1-1 encapsulation, has been dictated by double *Poisson* distribution, which yields only ~5% efficiency with common cell loading density. Such low efficiency makes it impractical for biological analyses at scale. Here, we demonstrate a passive 1-1-1 co-encapsulation microfluidic device that leverages close packing of cells with hydrodynamic sheath flow to achieve over two-fold improvement compared to the double *Poisson* model.

KEYWORDS: Droplet Microfluidics, Co-compartmentalization, Cell-pairing

INTRODUCTION

Characterization of cell-cell interaction events is essential in the development of immunotherapy, such as chimeric antigen receptor T-cell. Our preliminary results indicate that co-encapsulation of effector immune cells with their target enables real-time immunometabolic function assessment through glycolytic state determination via NADH autofluorescence [1]. Notable microfluidic devices to study cell-cell interactions include hydrodynamic traps that enable close contact between cells [2]. While droplet-based compartmentalization offers a throughput advantage without restriction to a fixed number of traps per device, the 1-1-1 efficiency for passive encapsulation is dictated by double *Poisson* distribution (~5% for common cell loading density). Despite inertial-based technique facilitates ordered cell trains to improve efficiency, this method is critically dependent on the properties of particulates, high cell loading density and flow rates [3]. Thus, we present a passive compartmentalization platform to address this shortcoming by leveraging hydrodynamics and close packing of cells to overcome double *Poisson* statistics (Fig. 1a). The presented technique achieves over two-fold improvement compared to the double *Poisson* model, which could expand the breadth of platform compatibility for various reagents and lower cell loading density.

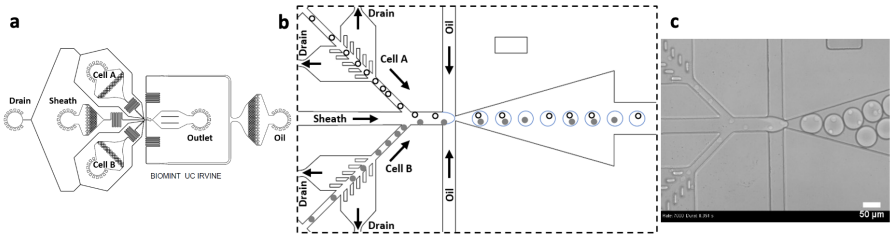

Figure 1: (a) Overall schematic of the passive encapsulation device. (b) Close-up schematic of the drainage and droplet generation junction. (c) Bright-field image of the co-encapsulation of K562 cells in monodispersed droplets.

THEORY

Each cell suspension enters the device and passes through a channel with width 20µm and height 40µm. This channel dimension facilitates self-assembly of a randomly dispersed cells into a centered single-file cell train. The cell train subsequently enters the drainage junction to attain higher on-chip cell density by draining a fraction of aqueous solution prior to entering the droplet generation junction.

Each drainage junction contains a pair of pillar arrays with 10µm gaps that are positioned on both sides of the channel at 45º angle backward with respect to the flow direction (Fig 1b). The bilateral draining flows are maintained at equal hydrostatic pressures and flow rates using the bifurcation channel design with equidistance. The effect of draining does not only increase on-chip cell density, but also effectively reducing the longitudinal spacing between cells. Thus, the λ value of its respective cell suspension also increases that results in improving the probability of 1-1-1 encapsulation. Given the laminar flow at low Reynolds number in microfluidic devices, both cell trains with shortened cell spacing flow in a single file as they merge with the sheath flow prior to collectively interfacing with oil phase at the nozzle to form droplets (Fig. 1c).

EXPERIMENTAL

The device with 40μm height was fabricated by soft-lithography. The aqueous phase consisted of 16% Optiprep, 1% BSA, 0.01% Triton X-100 in 1x PBS, and HFE7500 oil with 2% Fluorosurfactant was selected as the continuous phase. K562 cells were resuspended in aqueous phase at a volume fraction ranging from 2 to 2.5%. High-speed Phantom camera was used to assess the cell spacing and 1-1-1 encapsulation.

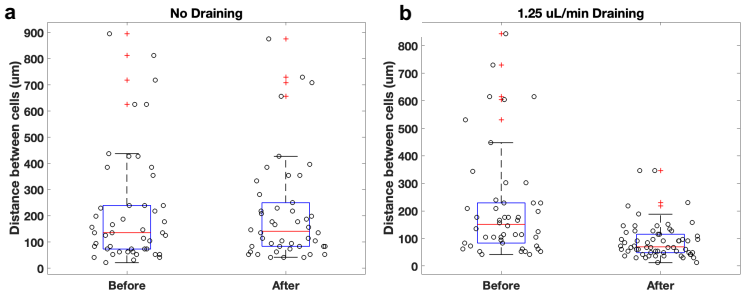

Figure 2: Cell spacing statistical assessment on drainage effect without (a) and with 1.25μL/min^{-1} drain rate (b).

RESULTS AND DISCUSSION

The cell spacing statistics in Fig. 2 reveals the comparison of draining for cell stream flowing at 3μLmin^{-1}. No significant difference was observed in cell spacing before and after drainage junction under the absence of draining (Fig. 2a). In contrast, the drain rate at 1.25μLmin^{-1} showed a substantial shortened cell spacing after drainage junction, in which the average spacing was reduced by nearly half (Fig. 2b). Such reduction in cell spacing minimizes the amount of empty droplets being produced during the co-encapsulation process.

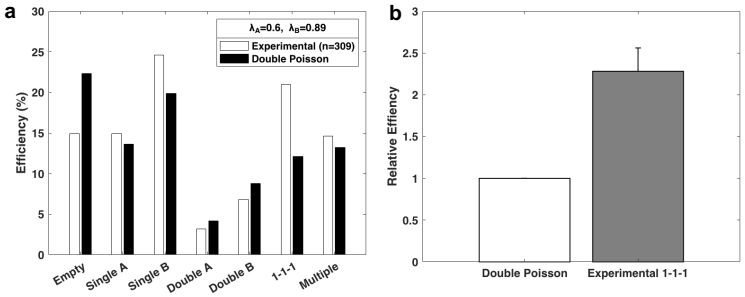

Figure 3: Encapsulation statistics on 1-1-1 device against double Poisson. (a) Distribution of cell occupancy per droplet. (b) Normalized efficiency of 1-1-1 encapsulation (N=3).

The device was applied to co-encapsulate K562 cells with two different densities. The droplet generation rate is optimized to be similar in the same order of magnitude to the rate of the cell arriving frequency for optimal results. With the optimized flow rates, the droplet generation rate was at around 980Hz. In Fig. 3a, the 1-1-1 encapsulation efficiency achieved as high as 21% of the droplets contain correct pairing, which has 2.8-fold improvement compared to the theoretical double *Poisson* statistics (7.4%). Furthermore, the distribution of the number of cells per droplet demonstrates that over 33% reduction in the amount of empty droplets and increment of single cell encapsulation for both loading densities. The co-encapsulation with cell densities of $\lambda<1$ on different devices were performed to yield an average of over two-fold improvement in 1-1-1 encapsulation efficiency, which exceeds the intrinsic double *Poisson* statistics and indicates robustness of the performance (Fig. 3b).

CONCLUSION

Our approach leverages a combination of close packing of cells and hydrodynamic sheath flow prior cell encapsulation in droplets. The fraction of empty droplet is substantially reduced, while the 1-1-1 encapsulation efficiency overcomes the double *Poisson* model by over two-fold. This simple, passive, and promising platform has the potential for broader range of cell pairing analysis for applications in immunotherapy.

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